Supplemental Figures and Methods for Slinger et al.
Discovery and validation of novel and distinct RNA regulators for ribosomal protein S15 in diverse bacterial phyla

Figure S1: Transcriptional analysis of Alphaproteobacterial and Actinobacterial RNAs.
To determine transcription start sites, we examined both the primary literature and the AREBA archive (A RNA Encyclopaedia for Bacteria and Archaea; Bacterial and Archaeal Transcriptome Meta-analysis Project, https://github.com/UCanCompBio/AREBA) to identify RNA-seq datasets for organisms that contain examples of the putative RNA. In this figure we display read depth plots from the AREBA archive (mapped RNA-seq data) from A: *Rhodobacter sphaeroides* [1] and B: *Mycobacterium tuberculosis* [2] displayed by the Artemis Genome Browser. This is paired in each case with the predicted RNA sequence and secondary structure annotation. In the case of *R. sphaeroides*, the transcription start site appears within the initially predicted 5’-most pairing element, calling into question whether this putative element is part of the RNA.
**R. radiobacter genomic rpsO genomic region**

AGATCCGGGAAGGCGAGTTCCAGCCGACAGGGTTTTGGCTAACCATACGGCGCGGAGGCGCTGTCAATCGCCTCTTTCAATTGCACCCGGGATAGTTTATAGGC

**Yellow:** 1 5’-RACE product

**Red:** 2 5’-RACE products

**Pink:** 4 5’-RACE products

**Green:** protein coding region

Predicted transcription start site based on R. sphaeroides transcriptome data.

**Figure S2:** 5’ RACE results for rpsO transcript in *Rhizobium radiobacter*.

**Supplemental Methods:**

**R. radiobacter RNA 5’-RACE**

Total RNA was extracted from log phase *R. radiobacter* cells grown in LB and 5’RACE performed using Invitrogen GeneRacer kit. Reverse transcription was conducted using a gene-specific primer (623-RradS15M11R: 5’-atcctttcttgtttaaggaaaaacggg), and the product PCR amplified with an oligo linker-specific primer (9-Forward5’RACE: 5’-gactggagcacgaggacactga) and gene-specific primer (647-RradS15M13R: 5’-gacgcctagagccgggatgtcgt) and gene-specific primer (647-RradS15M13R: 5’-gacgcctagagccgggatgtcgt). PCR product was cloned using TOPO-cloning kit (Invitrogen) and sequenced (Eton Biosciences) to identify the transcription initiation site of the rpsO transcript. The results of this analysis can be found in Supplementary Figure 2.

**Construction of ptrc-GFP plasmid**

To change the promoter, oligonucleotides encoding the trc IPTG-inducible promoter flanked by Xho I and EcoRI restriction sites at the 5’ and 3’ termini respectively (5’-gagctgttgacaattaatcatcgctgcgtataatgtgtggaattgtgagcgtataacaatt-3’), and its reverse complement were chemically synthesized (Eurofins MGW Operon), phosphorylated (T4 polynucleotide kinase, NEB), and annealed. The double-stranded DNA was inserted between the XhoI and EcoRI sites of digested pLac-thiMwt-tetA-gfpuv plasmid, replacing the pLac promoter. DNA fragment containing the RNA leader sequence with restriction sites EcoRI and SalI on the 5’ and 3’ ends respectively was PCR amplified from genomic template using gene specific primers. The PCR product was digested with EcoRI and SalI and inserted into pLac-thiMwt-tetA-gfpuv plasmid digested by the same enzymes, replacing the thiMwt riboswitch sequence. A DNA fragment encoding gfpuv replaced the existing gfpuv-tet reporter in this vector.
References: